NUTRITION OF THE SEA URCHIN, STRONGYLOCENTROTUS PURPURATUS [1]

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The gut of the purple sea urchin, *Strongylocentrotus purpuratus,* reveals a mass of algae in various stages of decomposition. Algae contain relatively small amounts of nutrients which are readily handled by enzymes ordinarily present in animals, but they possess galactans, alginic acid, agar and possibly some cellulose, none of which are digested by man or most animals. The urchins in the course of evolution may have developed enzymes which have enabled them to use these materials, or, like many of the ungulate herbivores, they might harbor bacteria, or, like the termites, they might shelter protozoans which perform this role for them.

The first study was therefore concerned with the role of the digestive enzymes present in the gut of the urchin. The second consisted of studies of the digestive action of the flora of the urchin gut. The third was concerned with the over-all nutritional economy of the sea urchin.

MATERIALS AND METHODS

Sea urchins were collected on the Monterey Peninsula at Yankee Point below Carmel Highlands and at Pescadero Point. A large number of urchins were planted just outside the Hopkins Marine Station to make them available for experiments requiring an occasional fresh specimen. While some of those transplanted died or disappeared, a fairly large number took hold and fed upon the prevalent coralline algae. Monthly studies were made on the urchins from Pescadero Point, because they could always be obtained even in rough weather, whereas those at Yankee Point sometimes became inaccessible in stormy weather. All the locations from which the urchins were taken were free of industrial waste and relatively free of sewage. The sea urchins brought in fresh monthly were kept in the laboratory in aerated rapidly running sea water. Even so, most of them aggregated near the top of the water in a tank, except when they wandered in search of food. Unless only a relatively small number of urchins were kept in an aquarium, they became unhealthy in time.

For determination of the sugar and nitrogen content in the body fluid, ten urchins were sacrificed the first day and ten the second after collection. The pH of body fluid was determined with a Beckman pH meter directly in the field, and

the pH of gut contents as soon as possible after opening a normal, healthy urchin in the laboratory.

The body fluid was withdrawn from an urchin by excising Aristotle's lantern and pouring out the contents of the coelom. The fluid was allowed to stand and the clot, containing wandering blood cells, was removed by filtration. Reducing sugars were determined in the filtrate by the method of Somogyi (1945, 1952). The non-protein nitrogen (NPN) in the coagulum-free filtrate of body fluid was determined by the standard Kjeldahl procedure, the proteins being first precipitated with trichloracetic acid (TCA).

Experimental

1. Structure of the digestive tract of the sea urchin

The mouth opening between the teeth of Aristotle's lantern leads through an oesophagus to the stomach-intestine which is suspended in the coelom by a mesentery. The oesophagus possesses well developed villus-like papillae which contain glands, perhaps producing mucus. The intestine has two turns; looking in at the mouth of the urchin the first turn is clockwise and the second, doubling back upon this, is counter-clockwise. The intestine is also lined by a glandular epithelium in which secretory cells are found, some forming glands possessing a body and a neck which opens into the digestive cavity. The connective tissue of this portion of the gut is inconspicuous and the epithelium appears to be bounded by the visceral peritoneum. Since the wall of the gut is so thin it is probable that absorption can occur readily through any part of it.

2. Feeding habits of the sea urchin

Over the last ten years, sea urchins kept in aquaria have been fed a great variety of foods. If starved, they were found to ingest almost anything offered them such as boiled eggs, boiled potatoes and vegetables, as well as fresh vegetables, but not leaves of geranium or *Pelargonium*. However, meat and fruits were taken in preference to vegetables. In nature the sea urchin feeds upon various algae (green, red and brown) as well as upon the "surf-grass," *Phyllospadix*. In local areas the diet may be largely restricted to the most abundant alga. In the laboratory, the sea urchins were usually fed the red alga, *Iridophycus flaccidum,* because of its availability and its acceptability to the urchin.

The ingested food apparently remains in the gut for a long period of time since during starvation in an aquarium, feces were ejected for two weeks, suggesting a very slow rate of digestion. When urchins brought in from the field were roughly handled, they eliminated considerable amounts of material for a short period. When slowly eliminated, the algae in the feces were found to be fairly completely decomposed and were heavily laden with bacteria. If feeding was continuous, so was defecation, and the algal pellets appeared to be less completely digested.

When an urchin which had been starved for some time in the laboratory was given food, it quickly fed to capacity with any material, algal or otherwise, upon which tests were desired. Starvation for two weeks was used as standard practice since in this time the gut will have become considerably, if not completely, cleared of contents.

Considering the low temperature of the water in which the sea urchins live, the

rate of digestion is not surprising. The water temperature for Moss Beach varied from 9.2 to 15.5 and at Stillwater Cove from 8.7 to 16.1° C. during the year. On the rare occasions when the urchins were exposed to sunlight at low tide the temperature may have risen considerably over this.

3. Digestive enzymes of the intestine

Since the normal food of the purple sea urchin consists of algae, the digestive enzymes most likely to be found in the gut are those which can handle the nutrients found in the algae. The protoplasm of algal cells, of course, contains protein, and floridean starch is stored in red algae. However, much organic material is present in red algae in the form of galactans or galactans mixed with other materials, e.g., agar and various gums. Enzymes which can handle proteins (proteases), starch (amylase) and the various substances peculiar to algae are therefore of special interest. Tests were made for each of these.

For extraction of enzymes, the gut contents were flushed out with sterile 3% NaCl, and the gut rinsed three times in the salt solution. The entire digestive tracts of several animals were then ground with crushed Pyrex glass in a mortar with a small amount of water, extracted with buffer solution and centrifuged. The buffer used in extraction was a 0.5 M McIlvaine buffer of pH 6.8–7.0. This pH was used because the first measurements of the pH of a sea urchin gut gave readings of 6.8–7.0. Subsequent measurements indicate that a pH of 7.2 to 7.3 is probably more nearly correct for a freshly opened gut. When the urchin is kept in the laboratory for a brief time the pH falls.

That a protease is present was easily demonstrated by mixing the gut extracts with casein, adding toluol to inhibit bacterial growth and determining the increase in NPN with lapse of time. Since some NPN appears in the control without casein, the data given in Figure 1 are for the differences between the two. The data demonstrate that the NPN rises rapidly after action of the enzymes on the protein.

That an amylase is present was shown by the appearance of reducing sugar in a sample of boiled starch mixed with gut extract (toluol as antiseptic). As seen in Figure 1 considerable reducing sugar appears after action of the enzymes in the extract on the boiled starch. As a control the gut extract was incubated without starch and tested for appearance of reducing sugar.

Agar and other gums contained in the algae are made up principally of polymerized galactose often combined with other substances. The ability of the sea urchin to digest agar was tested by adding the enzymes extracted from the gut to warm agar (about 37–40° C.) and mixing. The results, shown in Figure 1, may be considered negative since the very small change in reducing sugar concentration following exposure to the extracts is probably within the limits of error of the experimental method. The results for iridophycin, a galactan isolated from *Iridophycus flaccidum* by Hassid (1933), indicate that an iridophycase is present (Fig. 1). In all cases a control was incubated without the substrate and tested for reducing sugar. It would be desirable to test other substances peculiar to algae but they were not available in pure form for the tests.

4. Fauna and flora of the sea urchin gut

Ciliate protozoans occur in small numbers in the sea urchin gut, about ten to a hundred being counted per ml. of gut contents of several sea urchins. Several

species of *Entorhipidium* and *Lechriopyla mystax* have been identified (Lynch, 1929a, 1929b). Few were ever seen in division by Lynch; therefore they seem to represent a static population. The protozoans appear to ingest bacteria and small particles of well-disintegrated algae. While the activities of the protozoans, some of which live for several days outside the intestine, should be studied, it is unlikely that the protozoans contribute to the digestion of the algae because of their small numbers and their feeding habits.

Bacteria are present in the gut of the sea urchin in sufficient numbers to be of consequence. Almost every pellet of algal material in the second section of the intestine was found to be surrounded by a translucent membrane which, upon
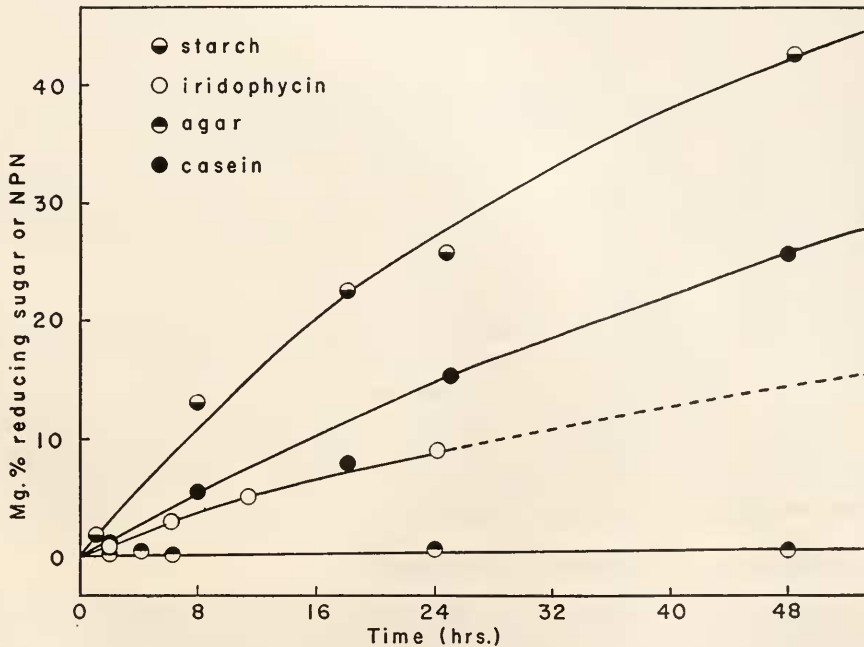

FIGURE 1. Rate of digestion of 1% casein by the extract of the intestine of the purple sea urchin at pH 6.8 and 30° C., and of 1% boiled starch, 0.1% agar and 0.02% iridophycin by the extract of the intestine of the purple sea urchin at pH 6.8 and 30° C.

microscopical examination, proved to be a film lined with coccus and rod-shaped bacteria. The films develop soon after the algal pellets enter the gut. When defecated the enveloped pieces were largely digested and no longer recognizable as algal pellets. An unenveloped piece of alga may become colorless but the cell walls remain intact and while no bacteria are seen within the walls, they occur around the cells damaged by the teeth. The envelope of mucilaginous material develops in the gut of the sea urchin, but not in cultures of the bacteria from the sea urchin gut growing on algae *in vitro*.

Since the enzymes from the sea urchin gut do not digest intact algal tissue, yet the latter disintegrates in the gut, it would appear that either the algae autolyze or

the bacteria digest them. Experiments eliminated the first possibility: algae in sea water under toluene did not autolyze. Attempts were therefore made to determine whether the bacteria from a sea urchin were capable of digesting the algae. An autoclaved sample of the red alga *Iridophycus* was inoculated with a loopfull of bacteria removed aseptically from the hind gut of a sea urchin. In one week at room temperature the algae had completely decomposed. The experiment was repeated several times.

Next, to test whether the bacteria in the lumen of the gut digest agar, a constituent of the cell walls of many algae, a sample of gut fluid was aseptically removed and transferred onto a 2% agar-sea water plate. The sample was poured over the plate or spread over it with a sterile glass spreader. Many colonies appeared within a few days. These were identified by the pits which they produced in the agar. Other colonies which did not form deep pits were detected by staining the agar surface with iodine. A clear area was noted around agar-decomposing colonies. The number of agar-digesting bacteria found in various tests was of the order of 10^6 per ml. of gut contents. For an urchin fed the red alga, *Iridophycus flaccidum,* the counts indicate 1.8, 2.1 and 1.4×10^6 bacteria and for one fed the brown alga, *Alaria marginata,* 1.7, 1.8 and 0.7×10^6. In a similar sea urchin, the total number of bacteria determined with a Petroff Hausser counting chamber was 2.6×10^{11}. In an urchin starved for a week after being fed *Iridophycus,* 1.0×10^6 agar decomposers appeared out of a population of 3.3×10^6.

Several individual colonies transferred to fresh agar-sea water plates were found to grow quite well on agar after a number of transfers, but some required additional nutrients or growth factors which could be supplied in the extract of algae or yeast extract. For culture purposes, the former was more convenient.

A loopfull of a pure culture of agar-decomposing bacteria was inoculated into a sample of autoclaved *Iridophycus flaccidum.* Within a week, at room temperature, the alga had completely disintegrated, therefore some of the agar-decomposing bacteria are capable of digesting this alga.

5. *Possible role of the intestinal flora in nutrition*

While some of the bacteria of the sea urchin intestine are capable of digesting the algae ingested, a symbiotic relationship between the bacteria and the sea urchin is not thereby proven. It is possible that the sea urchin maintains itself on the more readily available nutrients in the algae which it is capable of digesting—*i.e.,* the floridean starch and the proteins of the algae. The presence of enzymes capable of digestion of starch and protein supports this possibility.

On the other hand, the intestinal flora may render the nutrients in the cells of the algae more readily available to the sea urchin, by digesting the cell walls. However, it seems unlikely that the bacteria would spare the more generally utilizable nutrients such as starch and protein while selectively digesting the generally less available cell wall materials. It seems more likely that the enzymes present in the foregut of the sea urchin digest the more readily available materials in the algal cells before the bacteria have multiplied sufficiently to offer competition, and that the residue is then attacked by the bacteria which gradually decompose the algal cell walls.

The bacteria nevertheless may contribute to the host by digesting the structural

components of the algae and releasing some of the nutrients which can then be absorbed by the host into the body fluid. This would constitute a type of symbiosis. Sugar is mobilized (Table I) in the body fluid of a sea urchin soon after a meal of algae but this could be explained adequately as a result of digestion of starch in the algae by the amylases of the gut. Reducing sugars did not accumulate in cultures of the agar-digesting bacteria tested. It is possible that they are present only transiently in the intestine and might be absorbed to a small extent. Cultures of bacteria tend to become acidified and fatty acids may be demonstrated in the culture fluid and in the body fluid of the sea urchin. Identification tests indicate lactic acid, judging from the position of the spot in chromatographic analysis (Reid and Lederer, 1952) and the Friedemann-Graeser determination (1933).

TABLE I

Reducing sugar in the body fluid of the sea urchin after starvation for two weeks and refeeding or injection of glucose

Fed	Days fed	Weight of food ingested in grams	Wet weight of urchin	mg. % reducing sugar
Iridophycus (red alga)	0	0	37.5	0
	1	1.33	42.4	12.0
	2	2.07	57.1	4.5
	4	3.43	40.5	1.3
	8	6.86	41.9	1.0
Boiled potato	0	0	36.9	0
	1	2.89	47.8	47.0
	2	4.69	48.4	62.0
	4	4.55	38.5	1.7
	8	10.09	43.9	24.3
Glucose injected	Hours time lapse	Mg. glucose injected		% glucose withdrawn
	0	0.0	34.5	0
	1	1.0	54.2	64.5
	2	1.0	41.4	78.0
	4	1.0	39.0	95.8
	8	1.0	37.9	88.5

The nature of the relationship between the sea urchin and the bacteria could be ascertained if the bacterial flora of the intestine could be removed and replaced at will. Killing the flora by a meal of $CuSO_4$, as has been done with some vertebrates, kills the sea urchin as well. Defloration by high oxygen tensions is not practical since the bacteria present in the gut are only facultative anaerobes, not obligate like the termite protozoa. Raising sea urchin larvae aseptically to the adult stage is not likely to be successful with present techniques. A mixture of streptomycin and penicillin, 50 ppm in gelatin, was unsuccessful in killing the bacteria in the gut of the urchins tested. Therefore a crucial direct test for digestion in the absence of bacteria cannot be performed. Only circumstantial evidence can therefore be adduced at present, to support the possibility of symbiosis between the sea urchin and the bacteria, but it is not so overwhelming as to exclude other possibilities.

6. Reducing sugar in the body fluid and glycogen stores in tissues

Reducing sugar is usually present in the body fluid of the sea urchin as shown in Figure 2. The amount of sugar in individual cases varies from 0 to 13 mg. per cent. The sugar practically disappears after starvation (Table I). However, it is mobilized quickly in such an animal after a meal of algae or boiled potato (Table I). In the latter case the sugar rose from 0 to 62 mg. per cent in two days, the highest value ever obtained with this species of sea urchin over the course of a year of analyses. The intestinal amylases may be responsible for digestion but since bacteria are present in the gut their possible share in the digestion of the starch cannot be ignored.

Glucose injected into a starved sea urchin rapidly disappears from circulation (Table I). This suggests that the tissues take up glucose and other reducing
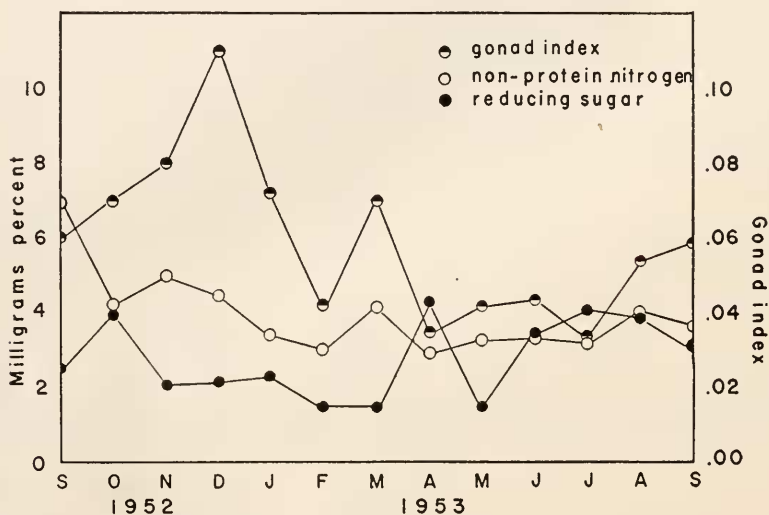

FIGURE 2. The gonad index (volume of gonad divided by the wet weight of the sea urchin) correlated with the average content of non-protein nitrogen (NPN) and reducing sugar in the body fluid of the purple sea urchin obtained monthly by analyses of twenty urchins.

sugars and store them in some insoluble form. Preliminary experiments have demonstrated glycogen in various tissues, but most appears in the intestine (Hilts and Giese, 1949). To determine the storage of glycogen a male sea urchin was drained of body fluid and the gut tissues were rinsed free of contents in distilled water and dried in an oven. The pulverized material, including the test, was extracted with alkali and the glycogen precipitated in alcohol. The glycogen present in a sample of the sea urchin was found by the method of Meyer (1943) to be 472 mg.% on the basis of dry weight, while 123 mg.% nitrogen was present. The amount of glycogen per unit weight of protoplasm was estimated as follows. Assuming that the protein of protoplasm contains all the nitrogen of the animal and that protein constitutes about 15% of the protoplasm, 3.84 units of glycogen are present per unit nitrogen or 0.62 unit of glycogen per unit protein, or almost 0.1 per cent glycogen in the protoplasm (muscle contains 1 per cent). The data

show that a considerable store of glycogen exists in the tissues and apparently the tissues draw upon this store for their respiratory activities. Stott (1931) found that in *Echinus esculentus* glycogen accumulated during growth of the gonads but declined just before the annual spawning in spring.

The avidity with which glucose is taken up following injection after starvation (Table I) suggests the possibility that the tissues of an urchin are starved for sugar. If this were true one might expect that addition of sugar would increase the respiration of excised tissues. Respiration of sea urchin intestine determined with the standard Warburg technique shows that this is not so. Regardless of whether glucose or yeast extract or both were added, the respiration of tissues freshly removed from a well-fed or a starved sea urchin and suspended either in sea water

TABLE II

Non-protein nitrogen (NPN) of the body fluid of the sea urchin
after two weeks starvation and refeeding

Fed	Days fed	Weight of food ingested in grams	Wet weight of sea urchin	mg. % NPN
Iridophycus	0	0	48.0	1.20
	1	2.73	56.6	4.30
	2	2.87	51.2	0.80
	4	3.28	45.9	3.75
Boiled egg albumin	0	0	28.9	3.19
	0	0	31.0	3.20
	1	3.03	35.3	6.67
	2	4.72	45.0	8.20
	3	2.54	44.3	3.75

or in the body fluid of the sea urchin did not increase significantly over the endogenous value. The average Qo_2 of digestive tissue was found to be about 0.7 ml./mg./hr.

7. Non-protein nitrogen in the body fluid

Non-protein nitrogen (NPN) is generally present in the body fluid of a sea urchin to the extent of about 5 mg. per cent. Data (averages) for monthly samples of groups of 20 urchins tested for a year are given in Figure 2. The NPN does not decrease markedly after starvation (Table II), but increases markedly after feeding with a high protein diet (Table II) rising in a day to twice the value for a control, and to an even higher concentration the second day. It falls off again on the third day.

The NPN seems to be regulated to some degree, since unlike the reducing sugar, it never falls to zero even after prolonged starvation. Nitrogen compounds stored in tissues must be liberated upon starvation to maintain the supply of soluble NPN. Storage of the nitrogenous compounds in the gonad is suggested, since the gonad of a sea urchin starved for a month is almost completely resorbed.

An attempt was made to determine the nature of the NPN by paper chromatography. The filtered body fluid from which proteins were removed with TCA

was treated with Duolite C3 resin in the acid cycle. The anions were washed out with distilled water and the amino acids were then separated from the cations by removal with NH_4OH and chromatographed in butyl alcohol (Redfield, 1953; Slotta and Primosigh, 1951). Only spots for alanine and glutamic acid were located. If other amino acids are present they occur in amounts too small to be detectable by even the very sensitive method used.

8. Nutrition and the reproductive cycle

Collections of this species of sea urchins over many years clearly suggest cyclic gonadal activity. Sperm are available practically all year but eggs are available only during a limited portion of the year. However, the gonads of both males and females undergo cyclic variation in volume. The cycle is probably different for each ecological habitat since differences in maturity were observed between the development of gonads of urchins at Yankee Point and at Pescadero Point. To ascertain the nature of the cycles, the gonads were removed monthly from twenty animals and their volume was determined by immersion in a graduate partially filled with sea water. The volume of gonadal tissues, divided by the weight of the urchin, designated the *gonad index*, was used for comparison of data which are given in Figures 2 and 3.

The growth of the gonads and the development of large numbers of gametes mean a synthesis involving the conversion of a considerable amount of nutrient to protein and nucleic acid. The amount of nitrogen in a gonad is large—a ripe testis containing 2.32% and a ripe ovary, 3.95% nitrogen per unit dry weight; therefore the nitrogen present in an animal probably increase several-fold during the breeding season. However the monthly determinations of the reducing sugar and the NPN in the body fluid given in Figure 2 provide no evidence of excessive mobilization or withdrawal of NPN or glucose during the breeding season.

DISCUSSION

Van der Heyde (1922) states that whereas the European sea urchin, *Echinus esculentus* is herbivorous and feeds largely on *Laminaria* and other algae, Neapolitan sea urchins such as *Sphaerechinus* and *Toxopneustes* may be carnivorous and may even capture various crustaceans. *Arbacia punctulata* appears to be omnivorous in nature since algae, brittle stars, hydroids and spicules of sponges are found in its gut (van der Heyde, 1922). *Strongylocentrotus droebachiensis* has similar feeding habits (Scott, 1901; Weese, 1926). The western purple sea urchin, *Strongylocentrotus purpuratus*, studied here was found to be quite omnivorous under laboratory conditions, but specimens found in the field invariably were feeding on any algae available or on the "surf-grass," *Phyllospadix*. The gut contents of hundreds of individuals examined appeared to consist entirely of algal pellets, although small amounts of animal food might have been missed since critical examination for this purpose was not attempted. *Lytechinus anamesus* also appears to be an algal feeder, judging from its depredations on algal beds used for commercial extracts (A. P. Steiner, personal communication).

Algae contain proteins and other constituents of protoplasm and may possess small amounts of sugar and starch. In place of the latter may be found sugar alcohols such as mannitol, dulcitol and sorbitol. However, the greater part of

the bulk of dry algae consists of the constituents of the cell walls and supporting materials. These are made up mainly of polysaccharides other than cellulose although the latter is sometimes found, *e.g.,* in some of the brown algae. Algin, found in the brown algae, is a condensation product of mannuronic acid, $(C_6H_8O_6)n$. Hassid (1936) has demonstrated sulfuric acid esters of galactans which make up to 40% of the dry weight of the red alga, *Iridophycus flaccidum.* When the sulfuric acid is split off, hydrolysis gives rise to pure galactose. Red algae also store floridean starch. Other compounds have also been demonstrated in algae but the knowledge of many is incomplete and the characterizations quite vague (see Blinks, 1951 for a review). Such lack of knowledge seriously hampers experiments on digestion of the algae by the sea urchin since it is generally necessary to use the entire algae rather than individual compounds.

Starch, glycogen, sucrose and maltose were found to be digested by ground-up intestine and contents of the sea urchin, *Echinus esculentus* (Roaf, 1908) and sucrose and protein by a similar preparation of *Strongylocentrotus droebachiensis* (Weese, 1926) and starch but not fats by *Sphaerechinus granularis* (Cohnheim, 1901; Scott, 1901). Weese (1926) failed to demonstrate lypolytic activity in *Echinus* but van der Heyde (1922) found evidence for it in *Arbacia*, the gut extracts of which developed acid when incubated with olive oil.

In the studies on *S. purpuratus* performed here, strong protease and amylase activity were demonstrated in ground, washed intestinal tissue, but no evidence was obtained for an invertase nor unequivocal evidence for an agar-decomposing enzyme, even though agar is ingested by hungry urchins. The sugar content of the body fluid of the starved sea urchin is not increased by a meal of agar. No increases in reducing sugar were observed nor were the algae decomposed in two weeks when they were mixed with the gut extract of the sea urchin and kept under pentachlorophenol. However, in one series of experiments in which the galactan iridophycin was mixed with the extract of the intestinal wall, an increase of reducing sugar was obtained, demonstrating the presence of an iridophycase in the gut. This experiment probably has more validity than those with algae, since in the latter case side reactions may occur by which the reducing sugar is bound. Other algal polysaccharides should be tested in a like manner, but none were available in pure form.

The possibility that bacteria might play a role in digestion has not been previously seriously considered although Weese (1926) observed bacteria present in a film about the algal particles in the gut of *S. droebachiensis.* The present study on *S. purpuratus* indicates that large numbers of bacteria are present, of which a considerable number are capable of digesting algae. The population of bacteria is even greater per unit volume than the figures given because for these determinations a 0.1-ml. quantity of gut contents was ground up with sand and an aliquot was counted or plated. The bacteria actually develop in films about the algal particles; therefore they occupy only a fraction of the volume sampled. The bacteria decomposing agar and other algal cell-wall materials might well be present in sufficient numbers to digest the algae in the gut of the urchin. Furthermore the food is retained in the intestine for a week to two weeks, a period of time adequate for even slow digestion.

It was not possible to determine whether the sea urchins could survive loss of their bacterial flora; therefore the role of the bacteria could not be defined. They

may be commensals which use what is left by the digestive enzymes of the sea urchin or they may be symbionts. The tentative conclusion is drawn that the sea urchin possesses enzymes which hydrolyze protein and starches of algae and perhaps slowly decompose some of the more resistant algal polysaccharides. In the latter action they may be greatly aided by the bacteria present in the gut.

Under normal nutritive conditions reducing sugar was almost always found in the body fluid of sea urchins taken in the field or feeding in the laboratory, the amount being greatest just after active feeding and least after starvation. Lang and MacLeod (1920) also report that little reducing sugar is present in the body fluid of the echinoderms which they tested, although Myers (1920) reported an unusually large content in *S. franciscanus*. The possibility exists that additional sugar is present in a combination with proteins or other nutrients as in some other invertebrates (Morel and Bellion, 1910).
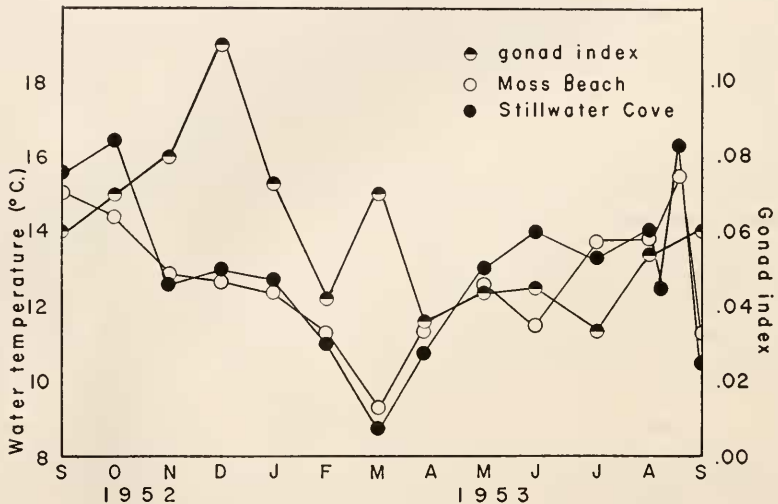

FIGURE 3. The gonad index (volume of gonad divided by the wet weight of the sea urchin) correlated with the water temperature during the year at Moss Beach, a habitat chosen because it lies on the open ocean, and at Stillwater Cover, a habitat chosen because it is sheltered.

The non-protein nitrogen (NPN) showed about the same variations throughout the entire year as the sugar but it was more closely regulated than reducing sugar during starvation. On starvation the NPN in the body fluid remained almost constant but the gonads shrank to a fraction of their former size. It would seem that the gonadal nitrogen was mobilized to maintain the NPN in the body fluid during starvation.

The gonads vary in size during the year and instead of a single growth period several were found in the monthly examinations made over a period of a year. The most striking was in December, but peaks occurred in March and June, presumably followed by spawning although all the spawning periods were not revealed by the monthly examinations. The gonadal cycle does not vary with temperature (Fig. 3) but may be related to cyclic changes in the algae providing

more food or more nutritious food at one time than at another. For lack of pertinent data in the literature it is impossible to test such a possible correlation, but on completion of a current study of algal populations in this region during the year, the necessary data may be available.

SUMMARY

1. The intestinal tract of the sea urchin, *Strongylocentrotus purpuratus,* consists of two loops, the first clockwise, the second counter-clockwise (as seen looking in at the mouth). Both loops are well supplied with glands.

2. Experiments present evidence for an amylase and a proteinase in the sea urchin intestine but none for enzymes capable of digesting entire algae or agar. However, the extract of ground gut was capable of digesting iridophycin, a galactan from the red alga, *Iridophycus.*

3. Agar-digesting bacteria are present in the second loop of the intestine in large numbers from 10^6 to 10^7 per ml. of gut contents. Total bacteria as determined by a count with a Petroff-Hauser counting chamber reach the value of 2×10^{11} per ml. They are largely confined to the pellicle which surrounds each particle of alga.

4. The bacteria inoculated from the gut of the sea urchin are capable of completely digesting the alga, *Iridophycus flaccidum,* in the course of a week. Many of the intestinal bacteria are capable of digesting agar. Pure cultures of agar-decomposing bacteria were isolated from the intestine.

5. The isolated bacteria grown on algae or agar do not liberate reducing sugars into the culture medium.

6. While it seems likely that the sea urchin obtains some nutrient from the activities of its intestinal flora, proof for this is lacking.

7. Some glycogen is stored in the tissue of the sea urchin and the body fluid normally contains a small amount of reducing sugar. No striking changes occur in the latter during the breeding season, but the sugar falls to zero or nearly zero on starvation. Glucose injected into the body fluid is removed by the tissues.

8. The body fluid of the sea urchin always contains a small amount of non-protein nitrogen (NPN), even after two weeks of starvation, and no striking change occurs during the breeding season.

9. The stimulus that sets off the increased effectiveness of the economy of the sea urchin resulting in the growth of the gonads and accumulation of nitrogenous compounds in them is unknown. After gonads reach their maximal size spawning occurs.

10. Several cycles of gonadal growth and spawning are suggested but the data were not collected at sufficiently frequent intervals to make this certain.

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